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CMA

CHEMICAL MANUFACTURERS ASSOCIATION

May 30, 1984

BY HAND

Mr. Gary Timm
Director
Office of Pesticides and
Toxic Substances
Environmental Protection Agency
Room 421-East Tower
401 M Street, S.W.
Washington, D.C. 20049

RE: Diethylene Glycol Monobutyl Ether

Dear Mr. Timm:

The Glycol Ethers Program Panel of the Chemical Manufacturers Association submits the enclosed studies on diethylene glycol monobutyl ether(DGBE). These studies include:

- Acute Oral LD_{50} (Eastman Kodak);
- Acute Dermal LD_{50} (Eastman Kodak);
- Six Weeks Repeated Dose (Eastman Kodak);
- Five Weeks Repeated Vapor Inhalation (Dow Chemical); and
- Short-Term Reproductive Toxicity (NIOSH)

The Panel will continue to collect available toxicological data on DGBE and share this information with you.

Please do not hesitate to call me at 887-1198 should you have any questions.

Sincerely,

Lori M. Ramonas, Ph.D.
Manager
Glycol Ethers Program

cc Dave Price

18-0293

TOXICITY STUDIES WITH DIETHYLENE GLYCOL MONOBUTYL ETHER

I. ACUTE ORAL LD_{50}

Eastman Kodak Company

April, 1984

TOXICITY STUDIES WITH DIETHYLENE GLYCOL MONOBUTYL ETHER

I. ACUTE ORAL LD_{50}

Introduction

Available data indicate that the glycol ethers, in general, have a low to moderate degree of toxicity. The majority of these compounds are only slightly irritating to the skin, though most are readily absorbed percutaneously. Eye contact produces moderate irritation. Prolonged or repeated inhalation of the vapors may cause irritation and adverse systemic effects; however, the relatively low volatility of these ethers should prevent exposure to toxic concentrations at normal temperatures.(1)

Several toxicity studies were designed in our laboratory to specifically study the toxicity of diethylene glycol monobutyl ether (DB). This report describes the results of acute oral LD_{50} studies in rats and mice.

Materials and Methods

The test compound was supplied by the Texas Eastman Company. An aliquot of each test sample was submitted to the Kodak Park Industrial Laboratory for identification of contaminants. Gas

chromatographic analyses showed that the test compound was > 99.5% pure. The chemical structure of DB was confirmed by infrared spectroscopy.

The test species were Charles River COBS, CD, BR male rats (150-200 g) and Charles River, COBS, CD-1 male mice (15-17 g). The animals of both species were received from the same supplier at the same time. All animals were quarantined and acclimated in our laboratory prior to being randomly assigned to the study. Two batches of both species were received approximately two months apart. The acute oral LD_{50} was determined in fasted and fed animals, with a period of two months between experiments. Each series of LD_{50} determinations (fasted and fed) in both species were conducted exactly the same except for the fasting prior to dosing and the batch of animals used.

Twenty-five animals of each species were divided into five dose groups of five rats or mice each. The doses given were calculated on a mM/kg basis for the purpose of comparison with other compounds and ranged from 10.5 to 168 mM/kg progressing by a factor of two. Dose administration was by gavage, undiluted, using a glass syringe fitted with a polypropylene catheter. The animals were individually housed in suspended wire-bottom cages. Water was available ad libitum and except for the removal of the food from the fasted animals 16-20 hours prior to treatment, food was available ad libitum.

General appearance and activity, pharmacologic and toxicologic signs and mortality were checked twice daily except on weekends and holidays. The appearance of stools and urine on the trays was noted and individual body weights were done prior to dosing and at the end of the two week observation period.

Animals that died during the study and all survivors were necropsied and examined for gross pathology. The survivors within a particular compound group were necropsied by the same prosector beginning with the high dosed animals and proceeding to the low dosed groups. The LD_{50} with its 95% confidence interval was calculated using the method of Thompson and Weil.(2)

Results and Discussion

Acute oral LD_{50} values for DB in fasted male rats and mice and fed male rats and mice are presented in Table 1. These results are in agreement with reports in the literature which indicate that the mono ethers of diethylene glycol have a low degree of acute oral toxicity.(1)

In both species, DB was less toxic in the fed animals, and was more toxic for mice than for rats in both fasted and fed animals.

Clinical signs of toxicity for both fed and fasted animals of both species were inactivity, labored breathing, rapid respiration, anorexia, slight to moderate weakness, tremors, prostration and death. Deaths occurred from within one to four days following treatment. Hematuria was not observed at any dose level of DB in either species.

No compound-related effects were observed on gross autopsy.

References

1. Rowe, V. K., Derivatives of Glycols, Chapter XXXVI in Industrial Hygiene and Toxicology, Vol. II, 2nd Edition, F. A. Patty, Ed., pp. 1537-1592, Interscience Publishers, Inc., 1963.

2. Thompson, W. R. and Weil, C. S., On the Construction of Tables for Moving Average Interpolation. Biometrics 8:51-54, 1952.

Table 1. Acute Oral LD_{50}'s of Diethylene Glycol Monobutyl Ether in Fasted and Fed Rats and Mice

Animal	LD_{50} (mmol/kg)	LD_{50} (mg/kg)
Rat (fasted)	45.0 (32.1 - 63.2)[a]	7292 (5200 - 10238)
Rat (fed)	59.4 (40.1 - 87.9)	9623 (6496 - 14240)
Mouse (fasted)	14.9 (9.1 - 24.2)	2406 (1474 - 3920)
Mouse (fed)	34.1 (22.0 - 52.9)	5526 (3564 - 8570)

[a] 95% confidence interval

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TOXICITY STUDIES WITH DIETHYLENE GLYCOL MONOBUTYL ETHER

II. ACUTE DERMAL LD_{50}

Eastman Kodak Company

April, 1984

TOXICITY STUDIES WITH DIETHYLENE GLYCOL MONOBUTYL ETHER -

II. ACUTE DERMAL LD_{50}

Introduction

Available data indicate that the glycol ethers, in general, have a low to moderate degree of toxicity. The majority of these compounds are only slightly irritating to the skin, though most are readily absorbed percutaneously. Eye contact produces moderate irritation. Prolonged or repeated inhalation of the vapors may cause irritation and adverse systemic effects; however, the relatively low volatility of these ethers should prevent exposure to toxic concentrations at normal temperatures.(1)

Several toxicity studies were designed in our laboratory to specifically study the toxicity of diethylene glycol monobutyl ether (DB). This report describes the results of acute dermal LD_{50} studies in rabbits and acute skin irritation studies in guinea pigs.

The skin irritation studies in guinea pigs were done in the Toxicology Section, Health, Safety, and Human Factors Laboratory, Eastman Kodak Company.

The test compound was supplied by the Texas Eastman Company. An aliquot of the compound was submitted to the Kodak Park Industrial Laboratory for identification of contaminants. Gas chromatographic analyses showed that the test compound was > 99.5% pure. The organic structure of the compound was confirmed by infrared spectroscopy.

Male New Zealand white rabbits were purchased from Dutchland Laboratory Animals Inc., Denver Pa. The animals were randomly assigned to groups of five rabbits each using a computerized randomization process. Each animal was assigned a unique identification number and housed individually in a metal cage marked with the animals's number. Purina Laboratory Rabbit Chow® and drinking water were available ad libitum.

The rabbits were quarantined and acclimated to the laboratory conditions for at least three weeks prior to treatment. During this period each animal received a health status examination by a staff veterinarian.

The test compound was administered to the closely clipped, unabraded back of the animals. Depending on the volume to be administered, one or more 3 1/2 x 4 inch Werbil® Handi Pads were held in close

contact to the skin by an occlusive wrap of dental dam. The compound was injected under the wrap into the pad using a long stainless steel intubation needle. The edges of the dental dam were then sealed using adhesive tape. The doses were administered on a millimole per kilogram of body weight basis to facilitate the comparison of LD_{50}'s with other compounds. The following four dose levels were administered; 10.5, 21.0, 42.0 and 84.0 mmol/kg (1701, 3402, 6804 and 13608 mg/kg).

After 24 hours, the wrap was removed, the amount of any residual compound was estimated and the area was wiped with cotton to preclude further absorption.

All animals were observed twice daily for mortality and once a day for abnormal signs. Dermal responses were noted and scored on days 1, 3, 7, 10 and 14 using the method of Draize.[2] Individual body weights were recorded prior to dosing, on day 7 and at death or termination (day 14). Survivors were killed on day 14 with an overdose of sodium pentobarbital, exsanguinated and autopsied. Animals that died during the observation period were autopsied also. Gross pathology was recorded for all rabbits. The LD_{50} was calculated using the method of Thompson and Weil.[3]

Primary skin irritation was determined by application of the compound to the depilated abdomen of the guinea pig at a dose of 1, 5, 10 or 20 mL/kg under an occlusive wrap for 24 hours.

Results

The acute dermal LD_{50} of DB for the rabbit was 17.06 mmol/kg with a 95% confidence interval of 12.9 to 22.5 mmol/kg. This is equivalent to 2764 mg/kg, with a 95% confidence interval of 2090 to 3645 mg/kg.

Clinical signs of toxicity noted after treatment were death, anorexia, depression, tremors and prostration.

The skin reactions in the rabbit, evaluated and scored according to the Draize method, indicate that DB produced only slight irritation. When skin irritation was determined in the guinea pig by the standardized procedure used in our laboratory, DB produced only slight irritaion. Table 1 presents the dermal irritation response for DB in the rabbit and the guinea pig. The dose associated with the irritation is the highest dose given at which enough animals survived so that irritation could be evaluated.

Gross pathology at autopsy is summarized in Table 2. Some evidence for adverse effects on the kidneys was noted, particularly at the higher dose levels. Reddish colored fluid was observed in the urinary bladder of the second lower dose group. No such findings were noted at either of the two higher doses. Edematous and hemorrhagic lesions of the thymus were observed at the higher doses.

References

1. Rowe, V. K., Derivatives of Glycols, Chapter XXXVI in Industrial Hygiene and Toxicology, Vol. II, 2nd Edition, F. A. Patty, Ed., pp. 1537-1592, Interscience Publishers, Inc., 1963.

2. Draize, J. H., Dermal Toxicity Appraisal of the Safety of Chemicals in Food, Drug and Cosmetics, pp. 46-59, 1959.

3. Thompson, W. R. and Weil, C. S., On the Construction of Tables for Moving Average Interpolation. Biometrics 8:51-54, 1952.

Table 1. Skin Response of Rabbits and Guinea Pigs to Topical Application of Diethylene Glycol Monobutyl Ether

Species	Dose[a] (g/kg)	Irritation[b]
Rabbit	3.4	Slight
Guinea Pig	19.1	Slight

[a] Highest dose at which sufficient animals survived for evaluation.

[b] Irritation in rabbit according to Draize; guinea-pig according to our laboratory standard procedure.

Table 2. Summary of Gross Pathology Examination in Rabbits

Diethylene Glycol Monobutyl Ether

	- Dose (mmol/kg) -				
	10.5	21.0	42.0	84.0	Total
No. Rabbits Examined:	5	5	5	5	20
No. survivors to 14 days	5	1	0	0	6
Thymus					
Edematous			2		2
Edema & hemorrhage				3	3
Increased vascularity			2		2
Yellow discoloration		3			3
Lungs					
Brown discoloration		2			2
Liver					
Brown discoloration		2			2
Kidneys					
Enlarged		1	1		2
Discoloration (tan)		1			1
Pelvis discolored		2			2
Stomach					
Hemorrhage		3			3
Small Intestines					
Increased vascularity			1		1
Urinary Bladder					
Fluid, dark red		3			3
Skin					
Reddened areas			4	5	9
Thoracic Cavity					
Brown fluid		2			2
Fat					
Tinged - yellow		3			3

18-0295

TOXICITY STUDIES WITH DIETHYLENE GLYCOL MONOBUTYL ETHER

III. SIX WEEKS REPEATED DOSE STUDY

Eastman Kodak Company

April, 1984

TOXICITY STUDIES WITH DIETHYLENE GLYCOL MONOBUTYL ETHER

III. SIX WEEKS REPEATED DOSE STUDY

Introduction

The ethers of ethylene and diethylene glycol have been extensively used in industry for the past 15 to 40 years. Available data indicate that the glycol ethers, in general, have a low to moderate degree of toxicity. The majority of these compounds are only slightly irritating to the skin, though most are readily absorbed percutaneously. Eye contact produces moderate irritation. Prolonged or repeated inhalation of the vapors may cause irritation and adverse systemic effects; however, the relatively low volatility of these ethers should prevent exposure to toxic concentrations at normal temperatures.(1)

Several studies were designed in our laboratory to specifically investigate the toxicity of diethylene glycol monobutyl ether (DB). This report describes the results of a six week repeated dose (gavage) study in male rats.

Materials and Methods

The test compound was supplied by the Texas Eastman Company. An aliquot of each test sample was submitted to the Kodak Park

Industrial Laboratory to be analyzed for purity and verification of molecular structure. Gas chromatographic results showed that DB was > 99.5% pure. The molecular structure of the compound was confirmed by infrared spectroscopy.

Male, albino rats (CR, COBS®, CD, BR) with an average body weight of 235.7 ± 15.1 grams purchased from the Charles Rivers Breeding Laboratories at Wilmington, MA, were used in the study. The animals were quarantined and acclimated to our laboratory for two weeks prior to the start of the study. The treatment group consisted of 30 rats and the control group of 10 rats. The treatment group was further subdivided into three dose groups of 10 rats each.

Doses of 3564, 1782 or 891 mg of DB per kilogram of body weight equivalent to 1/2, 1/4 or 1/8 the acute oral (fasted) LD_{50} determined in our laboratory were administered undiluted by gavage, five days per week for six weeks. This schedule provided 29-33 doses over a 44 day period. The control animals were handled similarly to the treated rats except that they received a volume of distilled water, equal to the largest volume given a treated animal. All doses were recalculated weekly to adjust for changes in body weights.

The animals were housed individually in suspended wire cages and Purina Rodent Chow 5001® and water, via an automatic watering system, were available ad libitum.

Individual body weights were recorded on days 0, 3, 6, 13, 20, 27, 34 and 41 of the study. Individual animal feed consumption data were recorded at the time the animals were weighed.

Animals were observed daily, except on weekends, for clinical signs of systemic toxicity and for deviations from normal with respect to general appearance and behavior. The appearance of urine and feces on the dropping trays was noted. Mortality was checked daily.

Blood was drawn from the inferior vena cava just prior to autopsy for hematologic and serum clinical chemistry determinations. These determinations consisted of hemoglobin concentration, hematocrit, red blood cell counts, red cell indices, total and relative white cell counts, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, lactic dehydrogenase, urea nitrogen, creatinine and glucose.

Animals that died spontaneously were autopsied as soon as possible and moribund animals were euthanized with CO_2 and autopsied. Tissues were collected for histopathologic examination.

At termination, the survivors were killed by CO_2 inhalation and the following tissues were collected, fixed in 10% buffered formalin, processed by standard histologic techniques and examined by light microscopy: lung, heart, thymus, kidneys, liver, spleen, brain, salivary glands, stomach, cecum, colon, duodenum, jejuneum,

ileum, pancreas, esophagus, adrenal glands, pituitary, thyroid, parathyroid, trachea, mesenteric lymph nodes, testes, epididymides, prostate, seminal vesicles, coagulating gland, bone marrow, tongue and nasal cavities. Eyes were fixed in Zenker's solution. Prior to being sectioned for fixation the liver, kidneys, heart, testes, brain and spleen were carefully trimmed and weighed for organ/body weight comparisons.

Results and Discussion

Mortality

The disposition of all animals on the study is shown in Table 1. No significant compound related mortality occurred in any dose group.

Body Weight and Feed Consumption

Individual body weights and feed consumption data with means and standard deviations, are listed in Tables 2 and 3. These data are presented graphically in Figures 1 and 2. Only the high dose of DB produced significant reductions in body weight gain and feed consumption. The intermediate and lower doses of DB produced slight, statistically non-significant, reductions in mean body weight gain.

Hematology and Clinical Chemistries

The individual values of the hematologic and serum clinical chemistry determinations are presented in Tables 4 and 5, respectively. The major hematologic effects produced by the test compound were effects on the red blood cells.

Diethylene glycol monobutyl ether (DB) decreased hemoglobin concentration and total red cells at the high and intermediate dose levels. Calculated red cell indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), reflected abnormal red cell morphology (microscytosis, macrocytosis, hypochromasia). The high and intermediate doses of DB increased MCV and MCH and decreased the MCHC.

The high and intermediate doses of DB produced significant decreases in the glucose level. This change, though statistically significant, was only slightly different compared to the controls and its toxicologic significance is uncertain.

Organ/Body Weight Comparisons

The individual terminal body and organ weights are presented in Table 6.

Significant reductions in mean terminal body weights were seen at all dose levels of DB.

Absolute and relative spleen weights of the animals given the high and intermediate doses of DB were significantly increased. The high and intermediate doses of DB significantly increased the absolute and relative weight of the liver.

Other organ weight changes noted were a reflection of the decreased body weight gain seen in these groups.

Clinical Signs

The high dose of diethylene glycol monobutyl ether (DB) produced bloody urine and blood around the nose and mouth in one rat after 23 days on the study. Other clinical signs noted at this dose level were dyspnea, prostration, and unkempt hair coat. No clinical signs of toxicity were seen at the intermediate and low dose levels.

Gross and Histopathology

Blood was seen in the urinary bladder of some animals from the high dose group of DB that died prior to termination and dark, enlarged spleens were seen in some of the survivors. No abnormalities were noted in the intermediate and low dose animals of DB.

Histopathologic lesions are listed in Table 7. All doses of DB produced hyperkeratosis of the stomach and acanthosis of the stomach was seen in a few animals.

Splenic congestion and red pulp hypocellularity and hemosiderin-like pigmentation were seen at the high dose of DB.

Histologically, renal effects included hyaline droplet degeneration, proteinaceous casts, and hemosiderin in the proximal convoluted tubules. The proteinaceous casts and hemosiderin appeared to be compound related but may have been secondary to the hematologic effects. The hyaline droplet degeneration was also seen in all ten control rats. Thus, the significance of this finding is uncertain.

Conclusions

No spontaneous deaths were observed in any of the dose groups. Two animals receiving the high dose were euthanized because of their moribund condition.

A statistically significant depression in body weight (22%) and food consumption (20%) was observed for animals receiving the high dose. For the low and intermediate dose groups, body weight and food consumption were not significantly different from those of the control animals.

Significant compound related histopathology was found in the spleens of DB treated rats. This consisted primarily of splenic congestion in 6/10 rats at the high dose only. Red pulp hypocellularity, hemosiderin and extramedullary hematopoiesis were also present in some animals at the high dose.

Kidney effects included proteinaceous casts in the proximal convoluted tubules. Hyaline droplet degeneration was seen in all test groups and in all control animals and was not considered to be treatment related.

No effects were seen on absolute testes weight or on testicular histopathology. Organ weight effects included increased absolute and relative liver and spleen weights at the high and intermediate doses.

Hematologically, significant decreases in red cells and hemoglobin were seen after treatment with DB, but only at the high dose level. No effects on clinical chemistry were noted.

References

1. Rowe, V. K., Derivatives of Glycols, in "Industrial Hygiene and Toxicology, Chapter XXXVI, Vol. II, 2nd Edition, F. A. Patty, Ed., pp. 1537-1592, Interscience Publishers, Inc., 1963.

Table 1. Repeated Dose Study with Diethylene Glycol Monobutyl Ether in Rats - Disposition of Animals

Dose in mg/kg (mmol/kg)	Spontaneous Deaths	Moribund Sacrifice	Intubation Error	Killed at Term
3564(22)	0	2	4	4
1782(11)	0	0	2	8
891(5.5)	0	0	1	9
Control[a]	0	0	0	10

[a] Received a volume of distilled water equal to the highest volume given to a treatment group.

Table 2. Body Weights (g)

BODY WEIGHT — DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 22 MMOL/KG

RAT	DAY 0	DAY 3	DAY 6	DAY 13	DAY 20	DAY 27	DAY 34	DAY 41
181	237	221	248	290	279	321	330	335
182	229	226	256					
183	221	205	234	263	256	276	292	299
184	257	228	253	310	311			
185	240							
186	214	199	207	262	255			
187	241	246	181	259				
188	217	199	215	265	262	293	289	292
189	224	210	244	287	290	326	337	338
190	231							
AVG	231.1	214.8*	229.8*	276.6*	274.8*	304.0*	312.0*	316.0*
S.D.	13.0	16.5	26.4	19.3	22.1	23.7	25.0	23.9

BODY WEIGHT — DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 11 MMOL/KG

RAT	DAY 0	DAY 3	DAY 6	DAY 13	DAY 20	DAY 27	DAY 34	DAY 41
191	262	275	283	322	322	349	372	395
192	221	246	265	306	333	365	386	392
193	252							
194	245	256	278					
195	242	259	276	312	323	356	366	386
196	258	271	288	329	343	369	387	414
197	216	226	244	277	291	316	337	356
198	221	223	244	267	281	304	329	349
199	217	229	249	277	293	325	344	366
200	234	254	276	305	324	341	370	383
AVG	236.8	248.8	267.0	299.4	313.8	343.1	361.4	380.1
S.D.	17.5	19.2	17.2	22.9	22.4	24.7	22.1	21.7

BODY WEIGHT — DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 5.5 MMOL/KG

RAT	DAY 0	DAY 3	DAY 6	DAY 13	DAY 20	DAY 27	DAY 34	DAY 41
201	239							
202	230	244	260	300	319	346	370	380
203	228	236	255	290	306	343	357	374
204	206	219	237	260	266	288	306	320
205	241	257	277	317	322	361	381	401
206	235	246	264	296	307	340	353	371
207	235	254	273	307	316	348	369	383
208	240	263	282	321	334	378	402	421
209	218	234	251	284	290	322	342	351
210	221	235	254	293	311	341	353	371
AVG	229.3	243.3	261.4	296.4	308.1	340.8	359.2	374.7
S.D.	11.3	13.9	14.2	18.3	20.2	25.0	26.8	28.5

*Statistically significant $p \leq 0.05$

Table 2 (cont.). Body Weights (g)

BODY WEIGHT — CONTROLS

RAT	DAY 0	DAY 3	DAY 6	DAY 13	DAY 20	DAY 27	DAY 34	DAY 41
271	226	236	261	283	304	339	363	374
272	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
273	211	230	256	290	311	342	356	381
274	257	269	[illegible]	329	342	[illegible]	406	410
275	207	218	234	266	271	303	316	330
276	241	272	[illegible]	328	341	[illegible]	406	424
277	252	266	276	307	330	363	379	400
278	234	259	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
279	248	276	292	350	372	416	440	466
280	223	245	263	304	316	353	383	[illegible]
AVG	234 6	253 3	272 0	311 4	328 8	363 3	387 3	[illegible]
S D	17 3	19 6	18 6	25 1	29 1	31 3	36 3	[illegible]

Table 3. Feed Consumption (g/rat/day)

FEED CONSUMPTION DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 22 MMOL/KG

RAT	DAY 3	DAY 6	DAY 13	DAY 20	DAY 27	DAY 34	DAY 41
181	10 7	16 2	20 5	19 0	20 8	20 4	20 5
182	12.0	17 3					
183	9.0	15 0	[illegible]	18 1	18.0	18 5	18 6
184	10 0	15 0	[illegible]	[illegible]			
185							
186	8.7	10.8	[illegible]	16 1	18 6		
187	14.7	8 7	15 1				
188	7.0	12 0	15 1	17.6	18.3	18 5	18 4
189	11 7	17 0	21 0	20.3	20 8	20 8	20 4
190							
AVG	10.47*	14.00*	[illegible]*	18 90*	19.30*	19.53*	19.47*
S D	2 37	3 14	2 11	1 17	1 39	1 52	1 13

FEED CONSUMPTION DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 11 MMOL/KG

RAT	DAY 3	DAY 6	DAY 13	DAY 20	DAY 27	DAY 34	DAY 41
191	20 7	21 7	23 8	22 8	22 8	23 6	24 0
192	25 3	24.0	25 5	25.5	26 2	26 8	27 4
193							
194	16 3	17 2					
195	17 3	20 2	21 2	22 0	22 6	22 3	23 0
196	21 0	22 2	23 6	23 7	23.2	23.5	23 4
197	16 7	18 7	19 5	20 2	20 7	21 6	22 0
198	16 0	24 0	22 3	21 3	21 8	22 4	22 7
199	17 7	18 8	20 2	20 9	21 1	21 7	22 6
200	21 0	21 8	23 5	22 7	23 2	22 6	23 1
AVG	18 04*	21.18	22.47	22 43	22.70	23.11	23 65
S D	2 63	2 07	1 54	1 65	1.70	1.70	1.62

FEED CONSUMPTION DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 5 5 MMOL/KG

RAT	DAY 3	DAY 6	DAY 13	DAY 20	DAY 27	DAY 34	DAY 41
201							
202	20 0	20.0	22 4	22 4	23.0	23.4	23 6
203	19 7	21 5	22 8	22 7	23 1	23 3	23 5
204	21.7	20.6	23 5	23 8	20.6	23.9	24 1
205	21.7	23 3	24.7	24 1	24.4	24.4	24 9
206	22 0	22 5	23 5	23 7	22.4	22.4	23 7
207	22 3	23.2	24 0	23 0	23.9	23.9	23 9
208	21.7	23 3	23 5	24 0	24 7	25 6	26.7
209	20 7	20 3	21 5	21 3	21 6	21.9	22 1
210	20.7	20.7	[illegible]	22 8	23.0	23.0	23 3
AVG	21.17	21.73	[illegible]	22 62	23 20	23.37	23 59
S.D	0.92	1 36	1 26	1.13	1.71	1.60	1.59

*Statistically significant from controls $p \leq 0.05$

Table 3 (cont.). Feed Consumption (g/rat/day)

FEED CONSUMPTION — CONTROLS

RAT	DAY 3	DAY 6	DAY 13	DAY 20	DAY 27	DAY 34	DAY 41
271	20.7	22.0	17.8	19.4	20.6	21.4	22.1
272	21.3	23.2	20.2	25.3	25.8	26.3	26.7
273	18.0	18.8	21.2	21.9	22.4	23.2	23.7
274	24.0	25.2	25.3	24.6	25.0	25.1	24.7
275	21.3	20.0	20.6	20.0	20.3	20.4	20.5
276	23.7	22.8	24.5	24.0	23.9	24.2	24.4
277	22.3	22.3	22.8	22.5	23.2	23.2	23.4
278	21.3	21.3	21.0	22.0	22.4	23.2	23.6
279	24.7	25.2	27.1	27.1	27.6	27.9	28.1
280	22.0	21.8	22.7	22.8	23.1	23.4	23.7
AVG	21.93	22.26	22.55	22.76	23.43	23.83	24.09
S D	1.93	2.02	2.78	2.36	2.26	2.20	2.14

Table 4. Hematology

HEMATOLOGY — DIETHYLENE GLYCOL MONOBUTYL ETHER (DGE) 22 MMOL/KG

RAT	HB	C.V	RBC	MCV	MCH	MCHC	WBC	POLY	LYMPH	EOS	MONO	BASO	BAND
181	11.9	30	5.96	83.9	20.0	23.8	12900	20	75	2	3	0	0
183								25	69	0	6	0	0
188	12.7	51	6.23	81.9	20.4	24.9	12700	11	88	0	0	0	0
189	13.9	46	6.87	67.0	19.7	29.3	9700	13	86	0	1	0	0
AVG	12.76*	49.0	6.353*	77.60*	20.03*	26.00*	11766.7	17.3	79.5	0.9	2.5	0.0	0.0
S.D.	0.80	2.6	0.467	9.23	0.35	2.91	1792.6	6.4	9.0	1.0	2.4	0.0	0.0

HEMATOLOGY — DIETHYLENE GLYCOL MONOBUTYL ETHER (DGE) 11 MMOL/KG

RAT	HB	C.V	RBC	MCV	MCH	MCHC	WBC	POLY	LYMPH	EOS	MONO	BASO	BAND
191	13.3	61	7.36	55.7	18.1	32.4	17900	14	85	0	1	0	0
192	14.0	52	7.96	60.3	17.6	26.9	10700	14	78	0	7	0	1
193	13.8	52	7.85	65.2	17.6	26.5	11700	14	81	1	4	0	0
196	12.7	43	7.49	57.4	17.0	29.5	9600	16	74	4	5	0	0
197	13.8	50	7.81	64.0	17.7	27.6	10400	14	82	0	4	0	0
198	11.9	39	6.94	56.2	17.1	30.5	6900	16	82	0	2	0	0
199	13.4	46	7.47	61.6	17.9	29.1	8900	12	86	0	2	0	0
200	13.2	48	7.67	62.6	17.2	27.5	11500	24	71	1	3	0	1
AVG	13.26*	46.4	7.569*	61.12*	17.32	28.75*	10950.0	15.5	79.9	0.8	3.3	0.0	0.3
S.D.	0.69	5.0	0.329	4.17	0.39	2.02	3296.2	3.7	5.2	1.4	1.7	0.0	0.5

HEMATOLOGY — DIETHYLENE GLYCOL MONOBUTYL ETHER (DGE) 5.5 MMOL/KG

RAT	HB	C.V	RBC	MCV	MCH	MCHC	WBC	POLY	LYMPH	EOS	MONO	BASO	BAND
202	13.9	45	8.46	53.2	16.4	30.9	11200	21	74	2	3	0	0
203	15.2	51	8.89	57.4	17.1	29.8	14000	20	76	0	4	0	0
204	14.2	46	8.62	53.4	16.5	30.9	9400	4	93	0	4	0	0
205	14.7	49	8.25	57.4	17.8	30.0	8900	26	67	1	6	0	0
206	14.1	44	8.41	52.3	16.8	32.0	7700	18	73	3	6	0	0
207	14.5	45	8.10	55.6	17.9	32.2	15400	9	91	0	0	0	0
208	14.2	45	8.20	54.9	17.3	31.6	13700	11	87	1	1	0	0
209	14.9	47	8.65	54.1	17.2	31.7	14600	4	95	1	0	0	0
210	13.8	44	8.13	54.1	17.0	31.4	10600	16	83	0	1	0	0
AVG	14.39	46.2	8.413	54.96	17.11	31.17	11677.8	14.3	82.1	0.9	2.8	0.0	0.0
S.D.	0.47	2.4	0.269	2.23	0.52	0.84	2036.7	7.8	10.0	1.1	2.4	0.0	0.0

*Statistically significant compared to controls $p \leq 0.05$

Table 4 (cont.). Hematology

HEMATOLOGY (Control)

RAT	HB	C.V.	RBC	HCV	MCH	MCHC	WBC	POLY	LYMPH	EOS	MONO	BASO	BAND
271	13.0	47	9.54	44.3	13.7	31.9	12700	12	81	0	7	0	0
272	14.4	46	8.09	36.9	17.8	31.3	11500	18	79	0	3	0	0
273	14.8	46	8.91	51.6	16.6	32.2	7900	12	88	0	0	0	0
274	14.1	45	8.46	51.2	16.7	31.3	8400	21	73	1	5	0	0
275	13.4	43	8.77	51.7	17.6	32.1	9400	15	81	1	3	0	0
276	15.7	48	9.58	50.1	16.4	32.7	10500	10	90	0	0	0	0
277	14.4	45	8.77	51.3	16.4	32.0	8700	19	76	3	2	0	0
278	15.4	49	8.89	55.1	17.3	31.4	9400						
279	14.9	47	8.46	50.6	17.6	31.7	13900	14	78	1	7	0	0
280	14.5	48	8.23	50.2	17.6	30.2	8400	19	77	1	3	0	0
AVG	14.86	46.9	8.771	53.60	16.97	31.68	10030.0	15.6	80.3	0.8	3.3	0.0	0.0
S.D.	0.97	1.4	0.497	2.98	0.70	0.68	1923.0	3.8	5.5	1.0	2.4	0.0	0.0

Table 5. Clinical Chemistries

CLINICAL CHEMISTRY DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 22 MMOL/KG

RAT	SGOT	SGPT	LDH	ALK PHOS	BUN	GLUC	CREATININE
181	68	36		244	12	83	0.50
183	85		424	184	19	86	0.55
188	65	29		278	17	97	0.50
189	95	37	681	171	11	128	0.50
AVG	78.3	32.7	652.5	219.3	14.8	98.5*	0.512
S.D.	14.2	4.7	323.1	90.4	3.9	20.6	0.025

CLINICAL CHEMISTRY DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 11 MMOL/KG

RAT	SGOT	SGPT	LDH	ALK PHOS	BUN	GLUC	CREATININE
191	73	23	719	78	9	125	0.45
192	80	35	387	162	16	92	0.60
195	55	23	709	245	14	81	0.55
196	68	30	418	188	10	93	0.40
197	75	35	347	171	12	94	0.45
198	71	26	472	257	12	96	0.40
199	72	29	565		11	107	0.45
200	91	37	314	193	13	102	0.50
AVG	78.1	30.1	493.9	187.7	12.1	110.1*	0.481
S.D.	9.9	6.0	151.7	63.0	2.2	31.0	0.064

CLINICAL CHEMISTRY DIETHYLENE GLYCOL MONOBUTYL ETHER (DB) 5.5 MMOL/KG

RAT	SGOT	SGPT	LDH	ALK PHOS	BUN	GLUC	CREATININE
202	78	25	432	309	13	144	0.50
203	116	33		190	14	102	0.60
204	77	27	316	149	10	121	0.50
205	65	23	269	163	16	129	0.60
206	84	17	490	141	14	132	0.55
207	75	13	362	174	13	109	0.50
208	67	24	309	185	11	126	0.50
209	67	28	455	190	13	175	0.50
210	69	37	322	232	12	134	0.50
AVG	77.4	25.2	392.0	192.6	12.9	132.4	0.528
S.D.	15.7	7.4	120.5	51.2	1.8	23.6	0.044

*Statistically significant compared to controls $p \leq 0.05$

Table 5 (cont.). Clinical Chemistries

CLINICAL CHEMISTRY CONTROLS							
RAT	SGOT	SGPT	LDH	ALK PHOS	BUN	GLUC	CREATININE
271	74	32	909	116	10	137	0.30
272	93	23	272	164	12	148	0.55
273	81	19	1088	129	11	121	0.45
274	65	32	302	190	11	144	0.50
275	71	28	336	223	12	133	0.60
276	109	23	1152	248	15	143	0.60
277	81	41	304	203	12	132	0.50
278	68	18	282	206	10	140	0.50
279	90	36	897	143	11	152	0.50
280	61	17	350	151	9	166	0.55
AVG	80.2	26.9	549.8	177.3	11.3	141.8	0.525
S.D.	15.0	8.2	354.5	43.3	1.4	13.5	0.049

Table 6. Organ/Body Weights

ORGAN WT. X BODY WT DIETHYLENE GLYCOL MONOBUTYL ETHER (DGE) 22 MMOL/KG

RAT	BODY	LIVER WT	%	KIDNEY WT	%	HEART WT	%	TESTES WT	%	BRAIN WT	%	SPLEEN WT	%
181	302.	14.76	4.87	2.05	1.01	1.04	0.34	3.18	1.05	2.04	0.68	1.22	0.40
183	261.	10.27	3.93	2.11	0.82	0.93	0.36	2.62	1.00	1.78	0.68	1.36	0.52
188	261.	12.20	4.67	2.42	0.73	1.23	0.47	2.42	0.93	1.79	0.69	1.46	0.56
189	304.	13.71	4.51	2.72	0.89	0.93	0.31	2.79	0.92	1.91	0.63	1.04	0.34
AVG	282.0*	12.720*	4.495*	2.340	0.912*	1.037	0.370*	2.752	0.975*	1.880	0.670*	1.270*	0.495*
S.D.	24.3	1.930	0.484	0.379	0.079	0.137	0.070	0.323	0.061	0.122	0.027	0.182	0.102

ORGAN WT. X BODY WT DIETHYLENE GLYCOL MONOBUTYL ETHER (DGE) 11 MMOL/KG

RAT	BODY	LIVER WT	%	KIDNEY WT	%	HEART WT	%	TESTES WT	%	BRAIN WT	%	SPLEEN WT	%
191	356.	10.37	2.91	2.03	0.82	1.15	0.32	3.43	0.96	1.77	0.50	1.38	0.39
192	367.	13.74	3.75	2.15	0.89	1.28	0.35	2.93	0.80	2.02	0.55	0.99	0.27
195	356.	12.08	3.41	2.17	0.89	1.22	0.34	3.22	0.90	2.02	0.57	0.80	0.22
196	372.	13.60	3.66	2.40	0.91	1.17	0.31	2.93	0.79	2.03	0.55	0.98	0.26
197	326.	11.15	3.42	2.34	0.78	1.00	0.31	2.81	0.86	1.97	0.60	0.79	0.24
198	303.	10.50	3.47	2.52	0.83	1.16	0.38	3.11	1.03	1.85	0.61	0.83	0.27
199	335.	11.75	3.51	2.05	0.85	1.11	0.33	3.16	0.94	2.06	0.61	0.82	0.24
200	331.	12.93	3.60	2.01	0.86	1.30	0.37	3.13	0.89	1.91	0.54	0.96	0.27
AVG	345.8*	12.117*	3.502*	2.159	0.834*	1.174	0.339	3.095	0.896*	1.936	0.564	0.944*	0.270*
S.D.	23.0	1.356	0.305	0.318	0.043	0.096	0.026	0.192	0.081	0.104	0.039	0.195	0.052

ORGAN WT. X BODY WT DIETHYLENE GLYCOL MONOBUTYL ETHER (DGE) 5.5 MMOL/KG

RAT	BODY	LIVER WT	%	KIDNEY WT	%	HEART WT	%	TESTES WT	%	BRAIN WT	%	SPLEEN WT	%
202	353.	11.20	3.17	2.81	0.91	1.21	0.34	3.11	0.88	2.13	0.60	0.75	0.21
203	341.	10.83	3.19	2.56	0.75	1.30	0.38	3.16	0.93	1.92	0.56	0.92	0.27
204	296.	8.00	2.97	2.13	0.72	0.91	0.31	2.66	0.90	1.95	0.66	0.64	0.22
205	370.	13.07	3.53	2.97	0.80	1.19	0.31	3.20	0.86	1.76	0.53	0.64	0.17
206	344.	10.75	3.13	2.69	0.78	1.40	0.41	3.08	0.90	2.03	0.60	0.57	0.17
207	394.	9.51	2.41	2.81	0.71	1.00	0.26	2.89	0.81	2.09	0.59	0.71	0.20
208	385.	12.74	3.31	2.60	0.83	1.48	0.38	3.19	0.82	2.15	0.55	0.79	0.20
209	325.	11.53	3.55	2.92	0.90	1.18	0.36	2.99	0.92	1.88	0.58	0.68	0.21
210	351.	11.27	3.21	2.93	0.83	1.15	0.33	3.39	0.97	1.98	0.56	0.75	0.21
AVG	349.*	11.044	3.191*	2.791	0.802	1.198	0.344*	3.074	0.888*	2.012	0.581*	0.717	0.207
S.D.	26.2	1.324	0.244	0.192	0.071	0.180	0.042	0.209	0.052	0.096	0.038	0.102	0.030

*Statistically significant from controls $p \leq 0.05$

Table 6 (cont.). Organ/Body Weights

ORGAN WT. % BODY WT (MALES)

RAT	BODY	LIVER WT	%	KIDNEY WT	%	HEART WT	%	TESTES WT	%	BRAIN WT	%	SPLEEN WT	%
271	351.	10.27	2.93	2.67	0.77	1.07	0.30	2.97	0.85	1.94	0.55	0.71	0.20
272	417.	12.24	2.94	3.43	0.82	1.33	0.32	3.11	0.75	2.01	0.48	0.73	0.18
273	353.	9.90	2.80	2.76	0.78	1.08	0.31	3.19	0.90	2.02	0.57	0.66	0.19
274	381.	11.23	2.95	2.80	0.73	1.15	0.30	2.95	0.77	1.96	0.51	0.84	0.22
275	312.	7.66	2.46	1.99	0.64	0.80	0.26	2.90	0.93	1.89	0.61	0.52	0.17
276	394.	9.84	2.50	2.52	0.64	1.29	0.33	3.20	0.81	1.93	0.49	0.55	0.14
277	367.	10.29	2.79	2.92	0.80	1.09	0.30	3.10	0.84	1.94	0.53	0.66	0.18
278	386.	11.12	2.88	2.89	0.75	1.23	0.32	2.81	0.73	2.06	0.53	0.53	0.14
279	432.	13.63	2.52	3.44	0.80	1.26	0.29	3.37	0.78	2.19	0.51	0.92	0.21
280	377.	10.73	2.85	2.40	0.77	1.12	0.30	3.15	0.84	2.15	0.57	0.61	0.16
AVG	377.0	10.587	2.803	2.834	0.750	1.142	0.303	3.075	0.820	2.009	0.535	0.673	0.179
S.D.	34.3	1.392	0.179	0.418	0.063	0.152	0.019	0.167	0.064	0.099	0.040	0.132	0.027

Table 7. Histopathology

Lesions/Dose	Diethylene Glycol Monobutyl Ether High	Inter.	Low	Control
Testes:				
Atrophy, seminiferous tubules	0/10	0/10	-	0/10
Epididymides:				
Degenerated spermatazoa	0/10	0/10	-	0/10
Hypospermia				0/10
Thymus:	0/9	0/10	-	0/10
Stomach:				
Hyperkeratosis	8/10	10/10	10/10	0/10
Acanthosis	1/10	0/10	2/10	0/10
Liver:				
Hepatocytomegally				0/10
Anisokaryosis				0/10
Lack of cytoplasmic basophilia				0/10
Spleen:				
Congestion	6/10	0/10	-	0/10
Red pulp hypocellularity	1/10	0/10	-	0/10
Hemosiderin	1/10	0/10	-	0/10
Kidneys:				
Proximal convoluted tubules, hyaline droplet degeneration	9/10	8/10	10/10	10/10
Proteinaceous casts	9/10	5/10	1/10	0/10
Hemosiderin	1/10	2/10	0/10	0/10

Figure 1

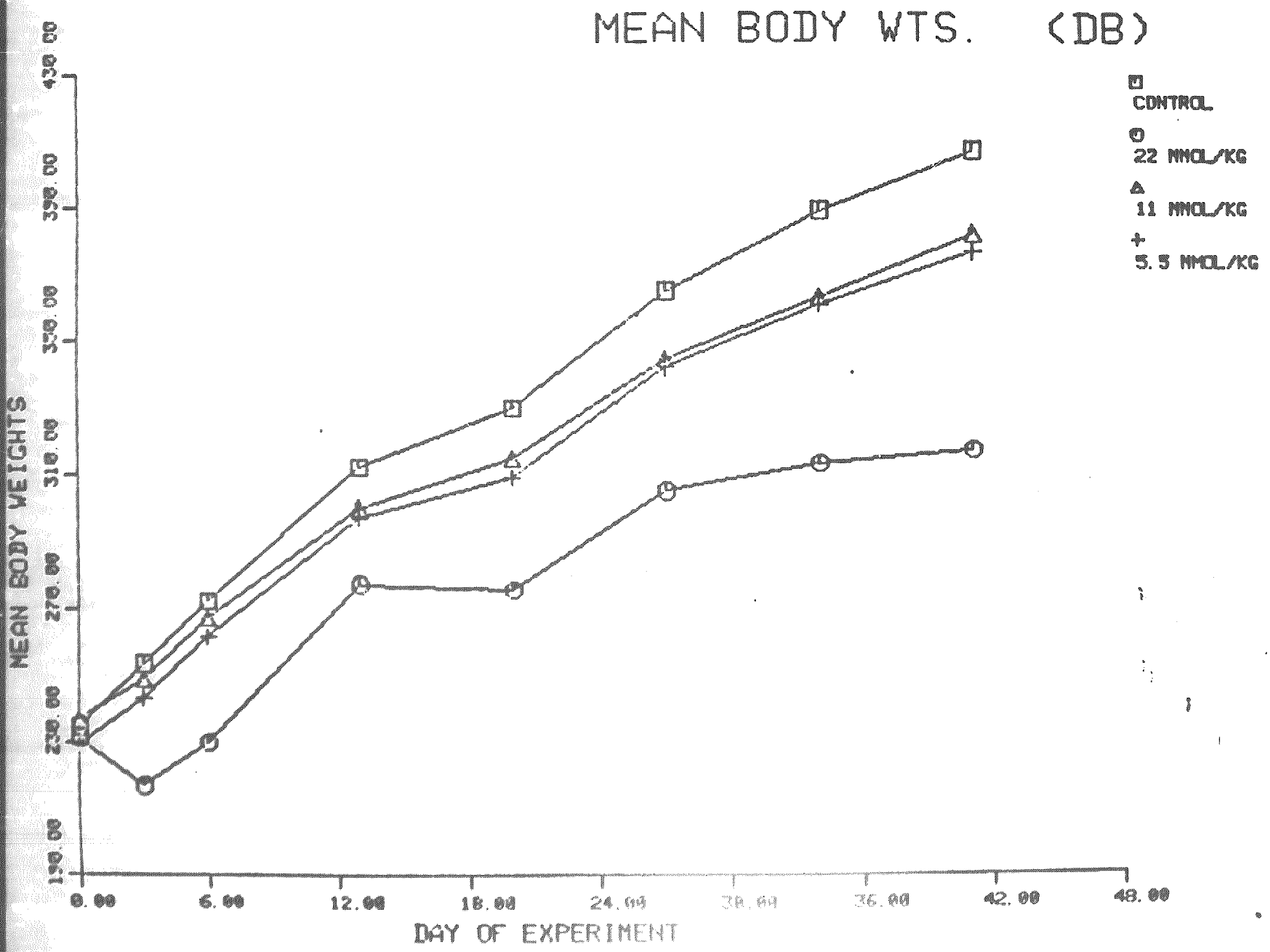
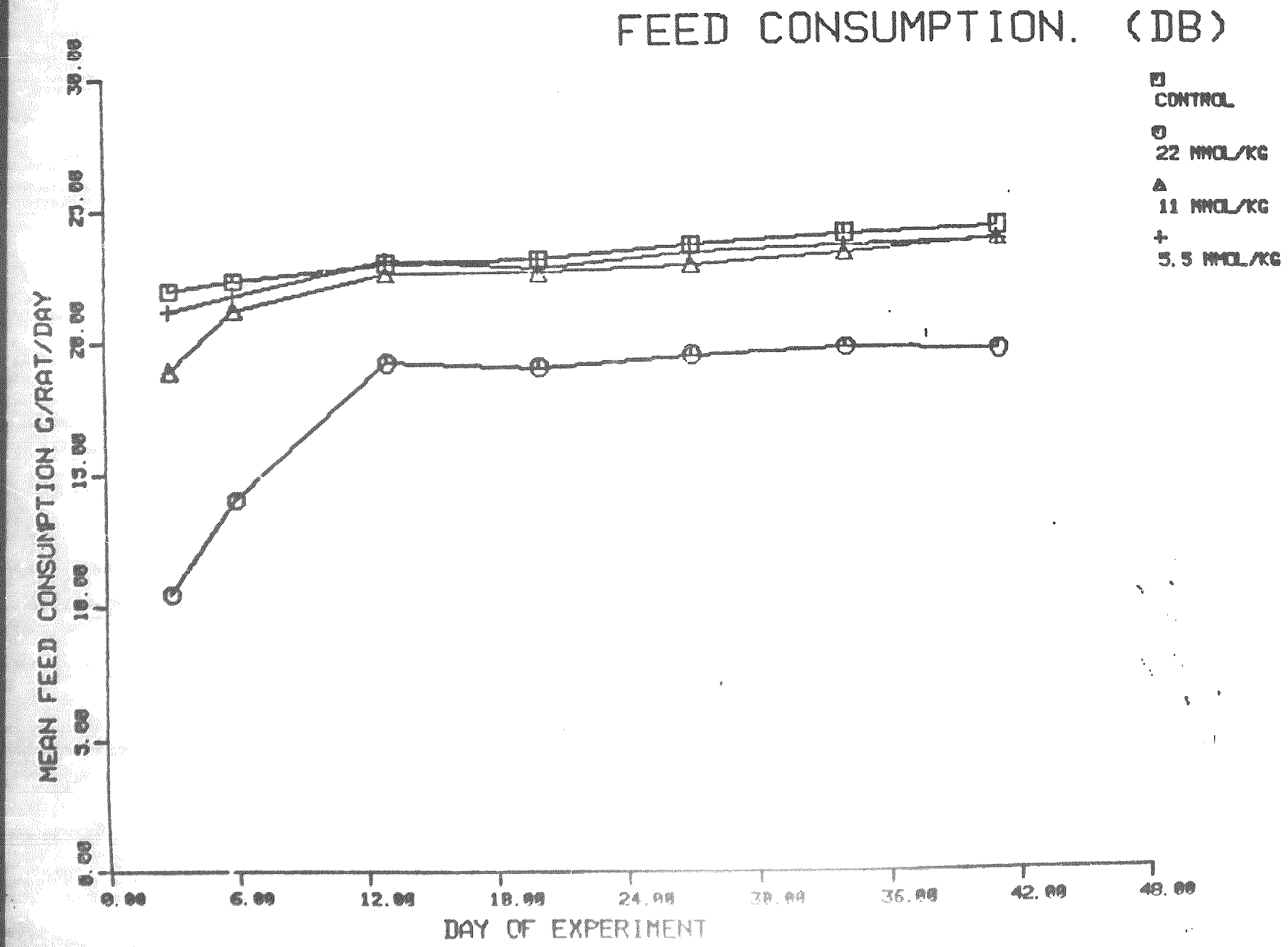

MEAN BODY WTS. (DB)
CONTROL
22 MMOL/KG
11 MMOL/KG
5.5 MMOL/KG
MEAN BODY WEIGHTS
430.00
390.00
350.00
310.00
270.00
230.00
190.00
0.00
6.00
12.00
18.00
24.00
30.00
36.00
42.00
48.00
DAY OF EXPERIMENT

Figure 2


FEED CONSUMPTION. (DB)
CONTROL
22 MMOL/KG
11 MMOL/KG
5.5 MMOL/KG
MEAN FEED CONSUMPTION G/RAT/DAY
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.00
6.00
12.00
18.00
24.00
30.00
36.00
42.00
48.00
DAY OF EXPERIMENT

18-0292

DOWANOL© DB: A 5-WEEK REPEATED VAPOR INHALATION STUDY IN RATS.

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Male and female Fischer 344 rats were exposed to 0, 2, 6 or 18 ppm DOWANOL DB (diethylene glycol monobutyl ether) for 6 hrs/day, 5 days/wk for 5 weeks. Parameters monitored were clinical observations, body weights, organ weights, hematologic parameters including red cell fragility, urinalysis, clinical chemistry, gross pathology and histopathology.

No treatment-related effects were found in male rats of any exposure group nor in female rats exposed to 2 ppm DOWANOL DB. Slight liver changes in female rats exposed to 6 or 18 ppm were observed. These changes were characterized microscopically as a slightly increased degree of hepatocyte vacuolization consistent with fatty change. Compatible with these microscopic changes, group mean relative liver weights were slightly increased, and paleness of liver was noted grossly in 3 of 10 females in the 18 ppm group. Since a very slight degree of vacuolization (apparent fat accumulation) was observed in the livers of the control and 2 ppm females, the minimal increase of vacuolization observed in the livers of the 6 and 18 ppm females was considered to have questionable toxicological significance. DOWANOL DB does not appear to pose an appreciable inhalation hazard because of its low vapor pressure and minimal toxicologic activity at vapor concentrations which can be attained.

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ABSTRACT

MALE AND FEMALE FISCHER 344 RATS WERE EXPOSED TO 0, 2, 6 OR 18 PPM DOWANOL® DB (DIETHYLENE GLYCOL MONOBUTYL ETHER) FOR 6 HRS/DAY, 5 DAYS/WK FOR 5 WEEKS. PARAMETERS MONITORED WERE CLINICAL OBSERVATIONS, BODY WEIGHTS, ORGAN WEIGHTS, HEMATOLOGIC PARAMETERS INCLUDING RED CELL FRAGILITY, URINALYSIS, CLINICAL CHEMISTRY, GROSS PATHOLOGY AND HISTOPATHOLOGY.

NO TREATMENT-RELATED EFFECTS WERE FOUND IN MALE RATS OF ANY EXPOSURE GROUP NOR IN FEMALE RATS EXPOSED TO 2 PPM DOWANOL DB. SLIGHT LIVER CHANGES IN FEMALE RATS EXPOSED TO 6 OR 18 PPM WERE OBSERVED. THESE CHANGES WERE CHARACTERIZED MICROSCOPICALLY AS A SLIGHTLY INCREASED DEGREE OF HEPATOCYTE VACUOLIZATION CONSISTENT WITH FATTY CHANGE. COMPATIBLE WITH THESE MICROSCOPIC CHANGES, GROUP MEAN RELATIVE LIVER WEIGHTS WERE SLIGHTLY INCREASED, AND PALENESS OF LIVER WAS NOTED GROSSLY IN 3 OF 10 FEMALES IN THE 18 PPM GROUP. SINCE A VERY SLIGHT DEGREE OF VACUOLIZATION (APPARENT FAT ACCUMULATION) WAS OBSERVED IN THE LIVERS OF THE CONTROL AND 2 PPM FEMALES, THE MINIMAL INCREASE OF VACUOLIZATION OBSERVED IN THE LIVERS OF THE 6 AND 18 PPM FEMALES WAS CONSIDERED TO HAVE QUESTIONABLE TOXICOLOGICAL SIGNIFICANCE. DOWANOL DB DOES NOT APPEAR TO POSE AN APPRECIABLE INHALATION HAZARD BECAUSE OF ITS LOW VAPOR PRESSURE AND MINIMAL TOXICOLOGIC ACTIVITY AT VAPOR CONCENTRATIONS WHICH CAN BE ATTAINED.

PURPOSE

THE PURPOSE OF THIS STUDY WAS TO EVALUATE THE EFFECTS OF DOWANOL® DB IN RATS EXPOSED FOR 5 WEEKS AT THE HIGHEST CONCENTRATION PRACTICALLY ATTAINABLE (APPROXIMATELY 18 PPM), ALONG WITH 2 LOWER EXPOSURE CONCENTRATIONS (2 AND 6 PPM). THESE RESULTS WILL BE USED TO HELP ASSESS THE POTENTIAL HAZARD FROM INHALATION OF DOWANOL DB.

GENERAL PHYSICAL PROPERTIES

PHYSICAL STATE	COLORLESS LIQUID
MOLECULAR WEIGHT	162.232 G/MOLE
DENSITY	0.955 G/ML
FLASH POINT (TCC)	222°F
BOILING POINT	230°C AT 760 MM HG
VAPOR PRESSURE	0.03[A] MM HG AT 23°C, 740 MM HG
MOLECULAR FORMULA	$C_8H_{18}O_3$
STRUCTURE	$HOCH_2CH_2OCH_2CH_2OCH_2CH_2CH_2CH_3$

ANALYSIS OF TEST MATERIAL

BOILING RANGE (°C)	229.9-232.8
H_2O% WT.	<0.01
ACID (% AS HAc)	0.002
APHA COLOR	5
SPECIFIC GRAVITY $\frac{25}{25°C}$	0.953

COMPOSITION

DIETHYLENE GLYCOL ISOBUTYLETHER	0.06%
DIETHYLENE GLYCOL	1.3 %
DIETHYLENE GLYCOL N-BUTYL ETHER	~ 98.6 % (BY DIFFERENCE)

NO OTHER IMPURITIES WERE DETECTED ABOVE 300 PPM.

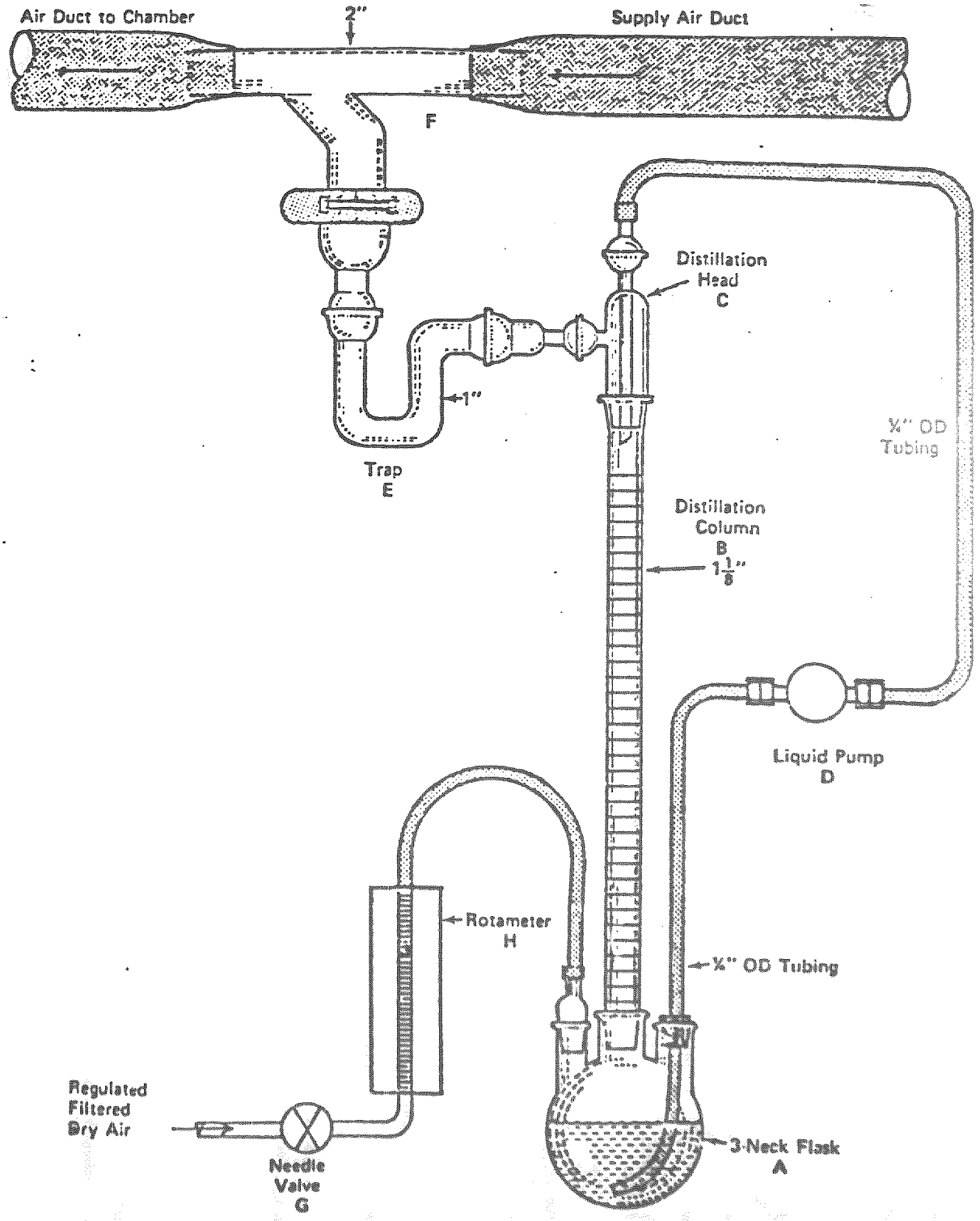
[A]CALCULATED BY ANTOINE EQUATION

STUDY DESIGN

ANIMALS	15 FISCHER 344 RATS/SEX/CONC.
EXPOSURE CONC	0, 2, 6 & 18 PPM, 6 HRS/DAY, 5 DAYS/WK FOR 5 WEEKS
EXPOSURE CHAMBERS	157 LITER ROCHESTER-TYPE STAINLESS STEEL AND GLASS WITH DYNAMIC AIRFLOW OF 25 LITERS/MIN.
PARAMETERS MONITORED	CLINICAL OBSERVATIONS
	BODY AND ORGAN WEIGHTS
	HEMATOLOGY
	RED CELL FRAGILITY
	URINALYSIS
	CLINICAL CHEMISTRY
	GROSS PATHOLOGY
	HISTOPATHOLOGY
STATISTICAL EVALUATION	BARTLETT'S TEST, $P < 0.01$
	ANALYSIS OF VARIANCE, $P < 0.10$
	DUNNETT'S TEST, $P < 0.05$
	WILCOXON'S TEST, $P < 0.05$

VAPOR GENERATION

15-PLATE PERFORATED PLATE DISTILLATION COLUMN[A]



[A]Potts, W. J. and Steiner, E. C. (1980). An apparatus for generation of vapors from liquids of low volatility for use in inhalation toxicity studies. Amer. Ind. Hyg. Assoc. Jl., 41:141-145.

CHAMBER ATMOSPHERE ANALYSIS

GAS CHROMATOGRAPH	VARIAN AEROGRAPH 2400
COLUMN	6' X 1/8" STAINLESS STEEL
PACKING	10% OV-101 ON 80/100 MESH CHROMOSORB W-HP
COLUMN TEMP	172°C
DETECTOR	H_2 FLAME IONIZATION AT 225°C

RESULTS

NO EFFECTS IN MALE RATS OF 2, 6, & 18 PPM GROUPS

NO EFFECTS IN FEMALE RATS OF 2 PPM GROUP

MINOR EFFECTS OF QUESTIONABLE TOXICOLOGICAL SIGNIFICANCE IN FEMALE RATS OF 6 & 18 PPM GROUPS

1. SLIGHT INCREASE OF HEPATOCYTE VACUOLIZATION CONSISTENT WITH FATTY CHANGE IN 6 & 18 PPM GROUPS OVER THE VERY SLIGHT DEGREE OF VACUOLIZATION OBSERVED IN THE 0 & 2 PPM GROUPS.

2. RELATIVE LIVER WEIGHT

   SLIGHT INCREASE IN 6 PPM GROUP

   STATISTICALLY SIGNIFICANT INCREASE IN 18 PPM GROUP

3. PALENESS OF LIVER IN 3/10 OF 18 PPM GROUP

LIVER WEIGHTS

SEX	EXPOSURE LEVEL (PPM)	N		FASTED BODY WEIGHT (G)	LIVER G	LIVER G/100 G
MALE	0	10	MEAN	228.2	7.43	3.26
			±S.D.	8.2	0.44	0.10
	2	10	MEAN	228.8	7.22	3.15
			±S.D.	16.8	0.69	0.14
	6	10	MEAN	225.6	6.93	3.07*
			±S.D.	15.1	0.69	0.14
	18	10	MEAN	227.7	6.92	3.04*
			±S.D.	13.3	0.47	0.06
FEMALE	0	10	MEAN	143.4	4.02	2.81
			±S.D.	5.7	0.25	0.10
	2	10	MEAN	140.9	3.97	2.82
			±S.D.	6.7	0.22	0.10
	6	10	MEAN	140.8	4.09	2.91
			±S.D.	3.5	0.18	0.11
	18	10	MEAN	138.9	4.13	2.98*
			±S.D.	6.1	0.19	0.11

*SIGNIFICANT DEVIATION FROM CONTROL MEAN USING DUNNETT'S TEST, $P < 0.05$.

CONCLUSIONS

DOWANOL® DB IS NOT AN APPRECIABLE INHALATION HAZARD BECAUSE OF ITS LOW VAPOR PRESSURE AND MINIMAL TOXICOLOGIC ACTIVITY AT VAPOR CONCENTRATIONS WHICH CAN BE ATTAINED.

PREPRINT

RESULTS OF TESTING FIFTEEN GLYCOL ETHERS IN A SHORT-TERM IN VIVO REPRODUCTIVE TOXICITY ASSAY

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Running Head: Short-Term Test of Fifteen Glycols

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KEY WORDS

Reproductive toxicity, teratology, screen, glycols, and glycol ethers

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RESULTS OF TESTING FIFTEEN GLYCOL ETHERS IN A SHORT-TERM *IN VIVO* REPRODUCTIVE TOXICITY ASSAY

ABSTRACT

Fifteen glycol ethers were investigated for their potential to cause adverse reproductive toxic effects using an *in vivo* mouse, screening bioassay. Pregnant mice were orally dosed once per day on days 7 through 14 of gestation at concentrations causing 0 to 41 percent maternal mortality. Reproductive endpoints included pup survival *in utero* (percent of live litters/pregnant survivors), pup perinatal and postnatal survival (number of live pups per litter, number of dead pups per litter, and pup survival to 2.5 days of age), and pup body weight statistics (weight at birth and weight at 2.5 days of age).

The study was conducted in two phases: a dose range-finding phase using non-pregnant female mice, and a definitive reproductive phase using time-mated mice. The range-finding phase sought to identify, for each chemical, the maternal LD_{10} as the target dose. However, based upon reproductive phase results, such an exact dose was impractical to achieve. Thus, a range from the LD_5 to the LD_{20} was considered a sufficient challenge dose that would not affect results due to high mortality, i.e., greater than the LD_{20}.

Glycol ethers were assigned to groups having different priorities for further testing based upon whether a sufficient challenge dose was administered and the degree of effects recorded for each chemical. These groups and chemicals

are: a) very high priority--triethylene glycol dimethyl ether (TriEGdiME); b) high priority--ethylene glycol (EG), ethylene glycol monomethyl ether (EGME), ethylene glycol monoethyl ether (EGEE), ethylene glycol diethyl ether (EGdiEE), and diethylene glycol monomethyl ether (DiEGME); c) middle to high priority--ethylene glycol dimethyl ether (EGdiME) and diethylene glycol dimethyl ether (DiEGdiME); d) middle priority--ethylene glycol monobutyl ether (EGBE), diethylene glycol (DiEG), diethylene glycol diethyl ether (DiEGdiEE) and triethylene glycol (TriEG); e) low priority--
diethylene glycol monoethyl ether (DiEGEE) and diethylene glycol dibutyl ether (DiEGdiBE). Diethylene glycol monobutyl ether (DiEGBE) was not administered at a sufficient challenge dose and should be repeated.

NIOSH does not regard these results as appropriate for labeling a compound as safe or unsafe. Instead they are suggestive, when considered along with other information on each chemical, of the urgency with which these chemicals should be considered for more detailed conventional testing.

INTRODUCTION

Conventional reproductive testing is expensive, involves complex scheduling, and requires the commitment of highly trained personnel. These intricate requirements, coupled with the existence of a large number of compounds that lack reproductive toxicity information, have created an urgent need for rapid, inexpensive methods of screening chemicals for reproductive toxicity. With this in mind, the National Institute for Occupational Safety and Health (NIOSH), in conjunction with the National Toxicology Program (NTP) in 1981 began to evaluate an *in vivo* screening test developed by Chernoff and Kavlock (1). For this test, pregnant mice are treated during organogenesis with high doses of the test chemicals. Females are then allowed to deliver their litters and the number of live-born pups, their birth weight, and growth and survival to 2-3 days of age are monitored. While the test is not appropriate for labeling a compound as safe or unsafe, it may serve to generate data useful in establishing priorities for conventional testing. It may also be useful for rapidly surveying structure-activity relationships. As part of the NTP evaluation of potential reproductive toxins, four contracts were awarded by NIOSH under which a total of 30 chemicals were tested. Fifteen of the chemicals were glycols or glycol ethers and the results of those tests are summarized here. Of these fifteen, three contractors tested four chemicals each and one contractor tested the remaining three (see Table 1). None were tested in more than one laboratory.

METHODS

All contractors used CD-1 mice purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Massachusetts) throughout these studies. Chemicals were evaluated in two phases: a preliminary dose-finding study in nonpregnant mice followed by the reproductive phase using time-mated females. In both phases chemicals were evaluated in blocks of 2 to 4 chemicals with a shared concurrent vehicle control group. Some blocks included only glycols, others included other chemicals. Only data on glycols are reported here. Chemicals were provided to contractors by NIOSH and were tested in blind, with the chemicals identified by an arbitrary code number. Table 1 summarizes the glycols tested and their abbreviations, structural formulas, chemical purity, and the laboratories that performed the investigations. DiEGdiBE was administered in corn oil due to its insolubility in water. Distilled water served as the vehicle for all other glycols tested. Dosage was by oral gavage in all instances.

The dose range-finding study was conducted at 5 dose levels using 10 mice, 6 to 8 weeks old, per treated or control group (except for diEGBE and diEGdiEE where the mice were 60 to 80 days old[1]). Upon receipt, mice were weighed and marked for individual identification, then formally randomized to treatment groups. Mice were group-housed, 5 per cage throughout the range-finding study. Standard laboratory rodent chow and untreated tap water were available ad libitum. Bedding of a type known not to induce microsomal enzymes was changed as needed or at least once per week. Oral doses were administered once daily for 8 consecutive days using a constant dose volume of

10 ml/kg body weight. Body weights were recorded on days 1 and 8 of the dosing period and on days 4 and 8 of an 8-day post-dosing observation period. Group mean or individual body weights taken on the first day of dosing were used to calculate treatment volumes over the entire 8-day dosing period. Survivors were sacrificed immediately following the last weighing on the 8th post-dosing day. All mice that died before that time were necropsied for evidence of dosing error as a cause of death. Based on the results of these dose-finding studies, the estimated LD_{10} dose was selected for the reproductive phase.

Reproductive studies were conducted in time-mated CD-1 mice, 6 to 8 weeks of age, orally dosed on days 7 to 14 of gestation (day 1 of gestation is the day on which a copulatory plug is observed). Mice were received on or before day 5 of gestation. On day 5 they were weighed and marked for individual identification, then formally randomized to treatment or control groups of 50 mice each. Test chemicals were administered at a single dose in a constant volume of 10 ml/kg body weight. Maternal body weights were taken on day 7 of gestation immediately before dosing, on day 18 of gestation, and on day 3 postpartum. The weight of the animal on gestation day 7 was used to calculate the dosage for the entire period. All mice were housed individually throughout the reproductive study. Food, water and bedding were provided as in the range-finding studies except bedding was not changed after gestation day 18.

Females were observed with minimal disturbances twice daily beginning on day 18 of gestation. As soon as possible after litters were delivered (within 12

hours), the number of live and still-born pups was recorded. Maternal body weight was recorded and all live pups were weighed together. Pups and their dams were then returned to nest boxes and were left undisturbed until 48 hours after the initial weighing, at which time the number of live pups, their total weight, and maternal body weight were again recorded.

Details of the statistical analyses varied from one contractor to another, but generally the procedures were similar. Body weight data were analyzed by analysis of variance. The proportion of surviving pregnant mice that gave birth to viable litters (1 or more live-born pups) was evaluated by the Fisher-Irwin Exact test. Numbers of live and still-born pups and percent survival to 2.5 days of age were analyzed by analysis of variance and the Student's t-Test.

RESULTS

Table 2 and Table 3 present the findings of the postnatal screen of the 15 glycol ethers. Because these chemicals were investigated by four independent laboratories at different times, and in some cases concurrently with other chemicals, seven control groups were used (some controls served more than one chemical). Control values were generally consistent across these seven groups. There was only one maternal death in all control groups and that was a result of a gavage error. Reproductive success in the controls ranged from 91 percent to 100 percent of all pregnant survivors. The average litter size ranged from 9 to 11 pups per litter. The percent pup postnatal survival (to 2.5 days postpartum) ranged from 98 to 100 percent. The average pup weight gain over days 1 to 3 postpartum ranged from 0.4 g to 1.1 g and the average pup birth weight ranged from 1.6 g to 1.7 g.

Nine of fifteen glycols tested affected the viable litter index. Pregnant mice treated with EGME, EGEE, EGdiME, diEGdiME, and triEGdiME produced no viable litters. Mice treated with EG, EGBE, EGdiEE and diEGME showed a significant reduction ($p<0.05$) in viable litters produced (41, 77, 11, and 16 percent viable litters produced, respectively). The remaining glycols and glycol ethers produced no effect on that reproductive index at the concentrations administered.

Postnatal observations varied widely in those groups with viable litters. EG and EGdiEE reduced the number of live pups per litter, increased the number of dead pups per litter, reduced pup survival, reduced pup birth weight, and reduced pup weight gain over days 1 to 3 postpartum. EGdiEE data were not analyzed statistically because of the small sample size. DiEGME significantly reduced ($p<0.05$) the number of live pups per litter and pup survival over days 1 to 3 postpartum. DiEGdiEE significantly increased ($p<0.05$) the number of dead pups per litter and significantly reduced ($p<0.05$) pup birth weight. DiEGdiBE increased ($p<0.05$) the number of dead pups per litter. DiEG reduced ($p<0.05$) pup weight gain over day 1 to 3 postpartum and triEG and diEGEE reduced ($p<0.05$) mean pup birth weight.

DISCUSSION

Because it is our intent to use this bioassay to screen chemicals for their potential to cause reproductive toxicity in pregnant females, it is necessary to employ clearly toxic doses. If a chemical is evaluated as a low priority, one wants to be relatively confident that it was tested at a sufficiently

severe challenge level. Clear maternal toxicity does not mean that reproductive toxicity follows. DiEG and triEG produced 4 percent maternal mortality; diEGdiBE produced 8 percent mortality; and diEGEE produced 14 percent mortality. None of these showed strong evidence of reproductive toxicity. In fact, diEGEE treatment did not adversely affect any of the reproductive indices.

As noted, the estimated maternal LD_{10} was chosen as the challenge dose to be used for the reproductive studies. In practice, mortality will vary somewhat, and a response in the LD_{5-20} range was considered acceptable in the reproductive phase. If reproductive effects are noted in the presence of more than 20Δ maternal mortality, the test probably should be repeated at a lower dose. Conversely, if there is no reproductive toxicity and less than 5Δ maternal mortality, the test probably should be repeated at a higher dose. This LD_{5-20} range, however, is only a tentative suggestion and further experience with this test may suggest other criteria for judging the appropriateness of the challenge dose.

The six endpoints examined for determining the priority of chemicals for further testing can be condensed into three levels of consideration. Most important is pup survival <u>in utero</u>, i.e., percent viable litters delivered by pregnant survivors. Of second importance is pup perinatal and postnatal survival, i.e., the number of live and dead pups per litter at birth, and pup survival over days 1-3 post partum. Final consideration is given to pup body weight endpoints, i.e., pup weight gain over days 1-3 and pup weight at birth.

Chemicals were assigned to groups having different priorities for further testing based upon whether a sufficient dose was administered (the maternal LD_{5-20}) and the degree of effects recorded for each chemical. TriEGdiME therefore has a very high priority for further testing because the administered dose of 4 percent maternal mortality was less than the LD_{5-20} and the results were profound, i.e., no viable litters delivered. Other chemicals deserving high priority include EG, EGME, EGEE, EGdiEE, and diEGME in that all were tested within the range of 5-20 percent maternal mortality and all showed a drastic reduction of viable litters. A middle to high level priority group would include EGdiME and diEGdiME because both produced no viable litters but they received a dose greater than the LD_{5-20} which could have influenced the results due to maternal toxicity effects. A middle level priority group would include EGBE, diEG, diEGdiEE and triEG. EGBE significantly reduced viable litters with no other effects when dosed at the upper limit of the range of acceptable mortality. DiEG, diEGdiEE and triEG mice received less than the LD_{5-20} dose but still produced some lesser effects: reduced pup weight gain for diEG; reduced pup weight gain and decreased number of live pups per litter for triEG; and an increased number of dead pups per litter plus a reduced pup birth weight for diEGdiEE. A low priority group would include diEGEE and diEGdiBE because the LD_{5-20} dose was achieved and only lesser effects were seen, i.e., a reduced pup birth weight for diEGEE and an increased number of dead pups per litter for diEGdiBE. DiEGBE mice did not receive a maternally toxic dose and no effects were found, thus diEGBE should be repeated until an LD_{5-20} dose is achieved.

It is important to note that, for the purposes of this screen, comparisons among chemicals are based upon maternal mortality, i.e., doses less than, greater than, or within the LD_{5-20} range. Comparisons as to the potential exposure hazard are not made. For example, although EG is designated to a higher priority group than EGBE, it may be that an individual is more likely to receive an 1,180 mg/kg dose of EGBE (LD_{20}) than he is to receive an 11,090 mg/kg dose of EG (LD_{10}). Thus EGBE may be more of a potential hazard than EG.

Previous investigations have shown several of the glycol ethers assessed in the current work to be teratogenic in traditional teratology test systems. EGME (designated in our high priority group) induced skeletal anomalies in the offspring of mice receiving, by oral gavage, as low as 31 mg/kg/day over days 7 through 14 of gestation (2). Gross (external) anomalies and the incidence of embryonic death were greatly increased in the offspring of mice receiving 250 mg/kg/day. In an inhalation study, 200 ppm EGME for 7 hours/day on gestation days 7 through 15 caused complete embryonic death in rats (3). Reduced fetal weights, skeletal and cardiovascular defects, as well as increased embryonic death occurred at both 50 and 100 ppm.

EGEE, also in the high priority group, induced skeletal anomalies, increased embryonic death, and decreased pup weight in offspring of rats treated by oral gavage on days 1 through 21 of gestation (4). Doses ranged from 12 to 327 mg/kg/day. Inhalation of 160 ppm EGEE for 7 hours/day on days 1 through 18 of gestation increased embryonic death and skeletal anomalies in rabbits (5). At 615 ppm complete embryonic death occurred. Offspring of rats receiving a 1.0 ml or 2.0 ml dermal application of EGEE on days 7 through 16 of gestation

showed greatly increased embryonic deaths, and cardiovascular and skeletal anomalies (2). Offspring of rats receiving 100 ppm of EGEE for 7 hours/day on days 7 through 13 or 14 through 20 of gestation showed altered behavioral patterns and altered brain neurochemical concentrations (7).

EGdiME, placed in our middle to high level priority group, administered to pregnant mice by oral gavage on days 7 through 10 of gestation caused increased embryonic death at concentrations of 250, 350, and 490 mg/kg/day (8). Skeletal and external anomalies occurred in the 490 mg/kg group. EGBE, in our middle priority group, caused no apparent adverse effect on the offspring of rats exposed to 200 ppm EGBE for 7 hours/day on gestation days 7 through 15 (3). DiEGEE, in our low priority group, caused no adverse effect on the offspring of rats exposed to 100 ppm for 7 hours/day on days 7 through 15 of gestation (3).

Some general statements can be made regarding structure activity relationships (Table 4). All mice receiving glycol ethers having terminal methyl groups, i.e., EGME, EGdiME, diEGME, diEGdiME, and triEGdiME produced few viable litters (0, 0, 16, 0, and 0 percent, respectively). Only EGEE and EGdiEE having terminal ethyl groups produced similar results (0 and 11 percent viable litters, respectively). The remaining ethyl ethers (diEGEE and diEGdiEE), the butyl ethers (EGBE, diEGBE, diEGdiBE), and the glycol ethers with terminal hydroxy groups (EG, diEG and triEG) did not produce such profound fetotoxicity. Maternal toxicity of the glycols was sharply increased by the addition of an alkyl group. EGBE was more toxic than EGME which was more toxic than EGEE. All three showed greater toxicity than EG. The diEG mono-

and diEGdi-alkyl ethers were more toxic than diEG, and triEGdiME was more toxic than triEG. The methyl ethers were generally more toxic than the ethyl or butyl ethers with the exception of EGBE.

We would caution again that neither NIOSH nor the NTP regard these results as definitive. Instead, they are suggestive, when considered along with other information on each chemical, of the urgency with which these chemicals should be considered for more detailed conventional testing. From the current available information on the glycol ethers, a correlation can be seen between the known positive teratogens (EGME, EGEE and EGdiME) and their results in this *in vivo* screen where these compounds were designated either in a high priority or middle to high priority group. Also, EGBE and diEGEE, which showed no reproductive toxicity in conventional tests, were given a lower priority ranking (middle or low group) following testing in this screen.

REFERENCES

1. Chernoff, N., and Kavlock, R. J. An *in vivo* teratology screen utilizing pregnant mice. J. Toxicol. Environ. Health 10:541-550 (1982).

2. Nagano, K., Nakayama, E., Oobayashi, H., Yamada, T., Adachi, H., Nishizawa, T., Ozawa, H., Nakaichi, M., Okuda, H., Minami, K., and Yamazaki, K. Embryotoxic effects of ethylene glycol monomethyl ether in mice. Toxicology 20:335-343 (1981).

3. Nelson, B. K., Setzer, J. V., Brightwell, W. S., Mathinos, P. R., Kuczuk, M. H., Weaver, T. E., and Goad, P. T. Comparative inhalation teratogenicity of four glycol ether solvents and an amino derivative in rats. Environ. Hlth. Perspect. (in press).

4. Stanger, E. G., Aeppli, L., Muller, D., Peheim, E., and Thomann, P. The toxicology of ethylene glycol monoethyl ether. Arzneim. Forsch. 21:880-885 (1971).

5. Andrew, F. D., Buschbom, R. L., Cannon, W. C., Miller, R. A., Montgomery, L. F., Phelps, D. W., and Sikov, M. R. Teratologic assessment of ethylbenzene and 2-ethoxyethanol. NIOSH contract report. Battelle Pacific Northwest Laboratory, Richland, WA, 99 pp (1981).

6. Hardin, B. D., Niemeier, R. W., Kuczuk, M. H., Mathinos, P. R., and Weaver, T. E. Teratogenicity of 2-ethoxyethanol by dermal applicaiton. Drug Chem. Toxicol. 5(3):277-294 (1982).

Neurotoxicology 2:231-249 (1981).

8. Uemura, K. The teratogenic effects of ethylene glycol dimethyl ether on the mouse. Acta Obstet. Gynaec. Jpn. 32(1):113-121 (1980).

FOOTNOTES

1. Because of the demand placed upon the single animal supplier by all four contractors, the initial requirement to use 60 to 80 day old mice was changed. This change required the use of the more readily available 6 to 8 week old mice.

Table 1
Glycol Ethers Investigated

Glycol	Formula: Structural	Formula: Empirical	Percent Purity	Contractors*
Ethylene Glycol (EG)	HO-Et-OH	$C_2O_2H_6$	99+	Inveresk
Ethylene Glycol Monomethyl Ether (EGME)	Me-O-Et-OH	$C_3O_2H_8$	99	Bioassay
Ethylene Glycol Dimethyl Ether (EGdiME)	Me-O-Et-O-Me	$C_4O_2H_{10}$	99+	MESA
Ethylene Glycol Monoethyl Ether (EGEE)	Et-O-Et-OH	$C_4O_2H_{10}$	99	Bioassay
Ethylene Glycol Diethyl Ether (EGdiEE)	Et-O-Et-O-Et	$C_6O_2H_{14}$	95	Borriston
Ethylene Glycol Monobutyl Ether (EGBE)	Bu-O-Et-OH	$C_6O_2H_{14}$	99	Bioassay
Diethylene Glycol (diEG)	HO-Et-O-Et-OH	$C_4O_3H_{10}$	97	Inveresk
Diethylene Glycol Monomethyl Ether (diEGME)	Me-O-Et-O-Et-OH	$C_5O_3H_{12}$	99	Bioassay
Diethylene Glycol Dimethyl Ether (diEGdiME)	Me-O-Et-O-Et-O-Me	$C_6O_3H_{14}$	99	MESA
Diethylene Glycol Monoethyl Ether (diEGEE)	Et-O-Et-O-Et-OH	$C_6O_3H_{14}$	99+	Borriston
Diethylene Glycol Diethyl Ether (diEGdiEE)	Et-O-Et-O-Et-O-Et	$C_8O_3H_{18}$	98+	MESA
Diethylene Glycol Monobutyl Ether (diEGBE)	Bu-O-Et-O-Et-OH	$C_8O_3H_{18}$	99+	Borriston
Diethylene Glycol Dibutyl Ether (diEGdiBE)	Bu-O-Et-O-Et-O-Bu	$C_{12}O_3H_{26}$	99+	Inveresk
Triethylene Glycol (triEG)	HO-Et-O-Et-O-Et-OH	$C_6O_4H_{14}$	99	Borriston
Triethylene Glycol Dimethyl Ether (triEGdiME)	Me-O-Et-O-Et-O-Et-O-Me	$C_8O_4H_{18}$	99	MESA

* Bioassay = Bioassay Systems Corporation, 225 Wildwood Avenue, Woburn, Massachusetts 01801
Borriston = Borriston Laboratories, Incorporated, 5050 Beech Place, Temple Hills, Maryland 20748
Inveresk = Inveresk Research International Limited, Edinburgh EH21 7UB, Scotland
MESA = Minority Enterprise Service Associates, 1156 South State, Orem, Utah 84057

Table 2
Glycol Ethers Results
Maternal Mortality and Pregnancy Success

Block	Glycol	Dose (mmol/kg)	Dose (mg/kg)	Maternal[a] Mortality (Percent)	Viable[b] Litters (Percent)
A	Control			0/50 (0)	31/32 (97)
	EGME	18.4	1400	7/49 (14)	0/30* (0)
	EGEE	40.1	3605	5/50 (10)	0/32* (0)
	EGBE	10.0	1180	10/50 (20)	24/31* (77)
	diEGME	33.3	4000	5/50 (10)	5/32* (16)
B	Control			0/49 (0)	30/31 (97)
	diEGBE	3.1	500	0/50 (0)	36/37 (97)
C	Control			0/50 (0)	42/42 (100)
	EGdiEE	25.0	2955	5/50 (10)	4/35* (11)
	diEGEE	41.0	5500	7/50 (14)	32/33 (97)
	triEG[c]	75.1	11270	2/50 (4)	36/36 (100)
D	Control			0/50 (0)	29/29 (100)
	EG[c]	178.9	11090	5/50 (10)	15/37* (41)
	diEG[c]	105.5	11180	2/50 (4)	33/36 (92)
E	Control[d]			0/50 (0)	45/45 (100)
	diEGdiBE[d]	9.2	2000	4/50 (8)	38/40 (95)
F	Control			0/50 (0)	41/45 (91)
	diEGdiEE	18.5	3000	0/40 (0)	35/41 (85)
G	Control			0/50 (0)	42/43 (98)
	EGdiME	22.2	2000	13/50 (26)	0/34* (0)
	diEGdiME	22.4	3000	20/49 (41)	0/27* (0)
	triEGdiME	19.7	3500	2/50 (4)	0/37* (0)

a Treatment-related deaths/number on test (percent mortality)
b Litters with 1 or more live-born pups/number of pregnant survivors (percent of pregnancies)
c Administered without dilution in volume of 10 ml/kg
d Corn oil used as the vehicle--all other groups used distilled water vehicle
* Differs significantly ($p<0.05$) from concurrent control by Fishers Exact Test

Table 3
Neonatal Observations

Block	Glycol	Dose (mmol/kg.)	Dose (mg/kg)	No. Live Pups Per Litter At Birth	No. Dead Pups Per Litter At Birth	Percent Pup Postnatal Survival	Pup Weight Gain (g) Over Days 1-3 Post Partum	Pup Birth Weight (g)
A	Control			10	0.1	100	0.4	1.6
	EGME	18.4	1400	no litters	-	-	-	-
	EGEE	40.1	3605	no litters	-	-	-	-
	EGBE	10.0	1180	10	0.2	95	0.4	1.5
	diEGME	33.3	4000	3*	0.6	31*	0.5	1.4
B	Control			10	0	99	1.1	1.6
	diEGBE	3.1	500	10	0	99	1.1	1.6
C	Control			10	0	99	0.9	1.6
	EGdiEE	25.0	2955	1**	2**	45**	0.6**	1.3**
	diEGEE	41.0	5500	10	0	98	0.9	1.5***
	triEG	75.1	11270	9	0	99	1.0	1.5***
D	Control			9	0.1	100	0.7	1.7
	EG	178.9	11090	2***	1.5***	40***	0.2***	1.4***
	diEG	105.5	11180	10	0.2	97	0.6***	1.6
E	Control			11	0	100	0.6	1.7
	diEGdiBE	9.2	2000	11	0.2***	100	0.6	1.7
F	Control			10	0.1	98	0.5	1.6
	diEGdiEE	18.5	3000	10	0.4*	97	0.5	1.5*
G	Control			10	0.3	98	0.7	1.6
	EGdiME	22.2	2000	no litters	-	-	-	-
	diEGdiME	22.4	3000	no litters	-	-	-	-
	triEGdiME	19.7	3500	no litters	-	-	-	-

* Significantly different ($p<0.05$) from concurrent control by analysis of variance
** Not tested statistically due to small sample size
*** Significantly different ($p<0.05$) from concurrent control by Student's t-Test

CERTIFICATE OF AUTHENTICITY

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Data produced 10 (Month) 7 (Day) 87 (Year)

Barbara Smith
Camera Operator

Place Syracuse (City) New York (State)

AMTEK corp